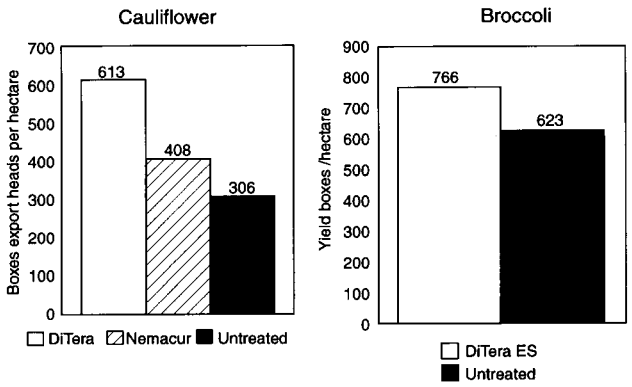

Figure 2. Yield benefits of treatment of cole crops at Salinas, California, USA against the sugar beet cyst nematode *Heterodera schachtii*. DiTera ES was applied as an 8″ band by sprinkler irrigation delivering 22 litres ha^{-1}. A. Cauliflowers; six replicate 0.3-ha plots per treatment. B. Broccoli; commercial trial involving four replicate 2 ha plots per treatment.

define the application parameters and to identify commercial benefits. Experimental details and the results are provided in the Figs 1 and 2.

6 CONCLUSIONS

The results demonstrate significant benefits in quantitative yield accompanied by nematode population reductions after application of DiTera treatments at critical stages in plant development. The product has now been commercialized in the cole crops and turf markets in the United States and the table grapes market in Mexico. Current development programs are focused towards addressing practical application parameters including timing, rates, methods of delivery, and new crops such as bananas, citrus and tobacco. Additionally, basic studies on the specific role of individual active molecules and the overall mechanisms involved in nematode management are under investigation. The unique product profile of DiTera offers an alternative, environmentally compatible option for plant parasitic nematode management in agricultural crops.

REFERENCES

1 Sasser JN and Freckman DW, A world perspective on nematology: The role of the society, in: *Vistas on Nematology*, ed by Veech JA and Dickson DW, Society of Nematologists, USA. pp 7–14 (1987).
2 United States Environmental Protection Agency. Protection of Stratospheric Ozone: Final rule. *1993 Federal Register*, vol 58, No 236, 10 Dec, 1993. pp 65018–65062.
3 Gintis B, Morgan-Jones G and Rodriguez-Kabana R, Fungi associated with several developmental stages of *Heterodera glycines* from an Alabama field soil. *Nematropica* **13**:181–200 (1983).
4 Devidas P and Crovetti AJ, Nematicidal preparations, *US Patent* 5,051,255 (1991).
5 Leath KT and Kandall WA *Myrothecium roridum* and *M verrucaria* pathogenic to root clover and alfalfa. *Plant Disease* **67**:1154 (1983).
6 Domsch KH, Gams W and Anderson T, *Compendium of soil fungi*. Academic Press, London. (1980).
7 Anon *DiTera, Biological nematicide*, Technical Bulletin, Abbott Laboratories. 28pp (1996).
8 Twomey P, Warrior P, Kerry BR and Perry RN, Effects of DiTera, a biological nematicide, on the aspects of the life cycle of the potato cyst nematode, *Globodera pallida*. *Proc Society of Nematologists Annual Meeting*, St Louis, USA. p 64 (1998).
9 Fernandez C, Rodriguez-Kabana R, Kloepper JW and Warrior P, Microbial antagonists to root-knot nematodes, increased by adding DiTera, a biological nematicide. *Proc Society of Nematologists Annual Meeting*, St Louis, USA. p 36 (1998).
10 Westerdahl BB, Anderson CA and Radewald JD, Natural products for nematode management on annual crops. *J Nematol* **27**:526 (1995).

The insecticidal activity of derivatives of the ionophore X-206

Katrin Fiebich, Aldo Hoermann, Michael Mutz, Anthony C O'Sullivan, Alfred Rindlisbacher and Fritz Struber*
Novartis AG, CH-4002 Basel, Switzerland

Abstract: The naturally-occurring ionophore X206, originally isolated for its antibacterial activity, also exhibits broad insecticidal and acaricidal activity. This summary reports structure/activity studies with X206 and 34 derivatives as well as mode of action studies. Although many compounds showed promising insecticidal activity, it was only of a contact activity nature; furthermore, the acute LD_{50} of the compounds in rats precluded further development of these compounds.

Keywords: X206 derivatives; ionophore; insecticide; acaracide; mode of action

The ionophore X-206 (Fig 1, **1**) was isolated in the early 1950s,[1] and its X-ray structure was determined in 1975 (Fig 2).[2,3] X-206 was originally isolated for its antibacterial activity,[1] but in 1980 Chugai patented its insecticidal and acaricidal activities.[4] More recently Grafe and Schlegel[5] found a new organism which produced X-206 and its broad insecticidal and acaricidal activities were confirmed by the Novartis screening laboratories (Table 1)

Although an LD_{50} of 17 mg kg^{-1} for X-206 in mice was reported,[6] the insecticidal activity was so promising that a derivatisation program was started.[7] This programme was guided by the ion-complexing abilities of X-206. In order to kill insects it is important for X-206 derivatives to be able to bind ions and transport them across cell membranes.[8] This is reflected by the good correlation of the insecticidal activity with the complexing ability of the compounds (Table 1). Derivatives at the C(1)-, C(9)-, or C(34)- O-atoms showed a complete or almost complete lack of insecticidal activity. It is known from

* Correspondence to: Fritz Struber, Novartis AG, Box No CH-4002 Basel, Switzerland.
E-mail: fritz.struber@pharma.novartis.com
(*Received 12 July 1998; accepted 25 November 1998*)

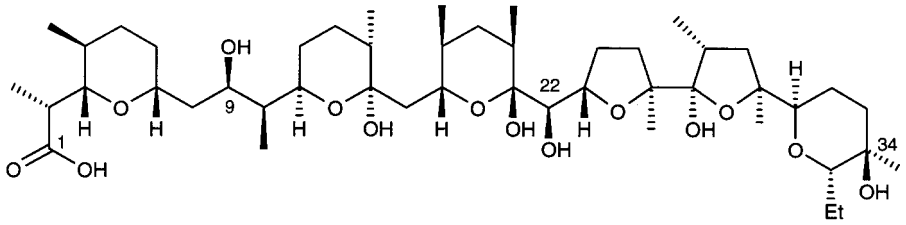

Figure 1. The structure of X-206 (**1**).

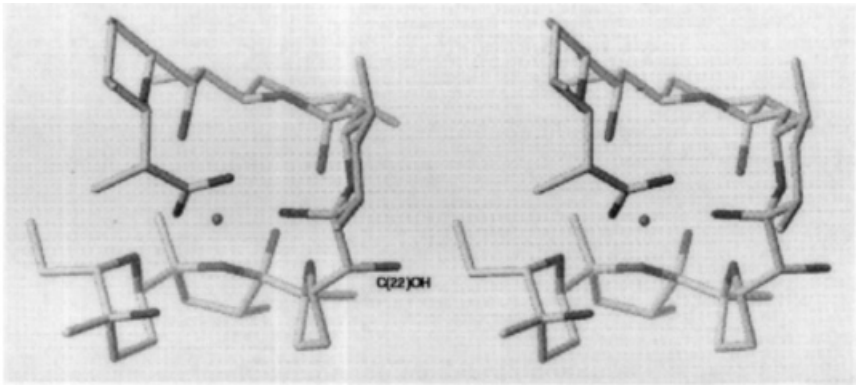

Figure 2. The X-ray structure of X-206 Na^+ salt.

the X-ray structure[2,3] (Fig. 1), that these O-atoms are involved in binding to the metal and are in a complex hydrogen-bonding network which helps to hold the molecule in its ion-binding conformation. Interrupting the binding of these *O*-atoms results in a weakening of the ionophoric properties of the whole molecule. The C(1)-OCH_2Ph derivative **2** showed no insecticidal activity in our tests and a greatly reduced ability to bind K^+ ions. It has a log $K_c(K^+)$ of 2.8 in comparison to that of 4.9 for X-206. Similarly the C(9)-OAc(**7**) and the C(34)-OBOM(**8**) derivatives did not bind Na^+ ions according to calorimetry, although X-206 had a log $K_c(Na^+)$ of 3.7. We suspect that the C(1)-ONH_2 compound **5** is insecticidally active because of its conversion back to X-206 (**1**) *in vivo*. Such compounds are known to be hydrolytically labile.[9]

On the other hand, the C(22)-OH group points out of the periphery of the tennis ball seam-like tertiary structure,[10] and derivatives at this position show both good K^+ and Na^+ binding ability and interesting insecticidal activity. For example, the C(22)-OAc (**15**) and the C(22)-OMe (**10**) derivatives have log $K_c(K^+)$ values of 4.4 and 4.8 respectively, which are similar to that of X-206. Similarly the insecticidal results were positive. Many of the derivatives at C(22) had good activity and many of these were much better than X-206 itself. Although the C(22) ethers **10** and **11** showed promising activity their synthesis required Meerwein's salts,[7] higher homologues of which are difficult to prepare.[11] Consequently, the C(22) esters were chosen to examine the results of a large series of compounds, as they could be prepared in one step directly from the X-206 potassium salt.[12] The straight-chain fatty acids showed interesting properties. The acetate derivatives (**15**) showed only moderate activity and the long-chain esters were virtually inactive, but the esters of intermediate size often showed very good activity. For example the *n*-butyrate (**17**) and iso-butyrate (**18**) esters showed such a good level of activity that we were hopeful of finding a development candidate among the X-206 derivatives. However, two results discouraged us from continuing work on this molecule. First, the acute LD_{50} in rats was found to be between 5 and 25 mg kg^{-1} both for X-206 and its *n*-butyrate ester **17**. Second, the X-206 derivatives seemed to have only contact insecticidal activity. This was shown by spraying bean plants before or after infestation with a mixed population of *Frankliniella occidentalis* Perg. All of the compounds tested were inactive when sprayed onto leaves before infestation, although good activity was found after spraying pre-infested plants.

In summary, it was shown that it was possible to modify the insecticidal activity through derivatisation in a rational manner, resulting in compounds with increased activity, but, in the compounds tested, toxicity remained a problem.

REFERENCES

1 Berger J, Rachlin AI, Scott WE, Sternbach LH and Goldberg MW, *J Am Chem Soc* **73**:5295–5298 (1951).
2 Blount JF and Westley JW, *J Chem Soc Chem Commun* (1975), 533.
3 Roey P van, Duax WL, Strong PD and Smith GD, *Isr J Chem* **24**:283–289 (1984).
4 Chugai Pharmaceutical Co Ltd, JP 81,118,008 (1980) (*Chem Abst* **96**:2997m (1982).
5 Graefe U, Stengel C, Schlegel R, Wiesner J, Ihn W and Fleck W, DD 298428 A5 920220.

Table 1. Ionophoric properties and insecticidal activities of derivatives of X-206

Cpd	*R*	LD_{80} [bc] (*mg litre*$^{-1}$)						
		log K_c [a] (K^+)	*log* K_c [a] (Na^+)	*H vir*	*P xyl*	*D bal*	*F occ*	*T urt*
1	—	4.9	3.7	c	50	25	50	50
2	C(1)OCH_2Ph	2.8	2.9	a	a	a	a	a
3	C(1)OpTol			a	a	a	b	b
4	C(1)OMe	2.6		a	a	a	a	a
5	C(1)ONH_2			12.5 gr	100	12.5	#	50
6	C(1)NHOH	—	—	a	a	c	b	c
7	C(9)OCOMe		—	a	a	100	b	b
8	C(34)OBOM		—	a	a	50	b	b
9	C(34)OCH_2Ph			a	a	a	b	50
10	C(22)OMe	4.8	3.6	25	b	50	100	50
11	C(22)OEt			25	50	50	100	25
12	C(22) = 0	—	—	a	a	a	b	c
13	C(22)-desoxy	4.5	3.9	b	a	<100	a	<100
14	C(22)-*epi*-OH			a	a	a	a	a
15	C(22)OCOMe	4.4	3.5	25	100	b	#	50
16	C(22)OCOEt			12.5	12.5	12.5	50	25
17	C(22)OCO*n*Pr			12.5	3	3	12	12.5
18	C(22)OCO*i*Pr			25	3	3	50	25
19	C(22)OCO*n*C_5H_{11}	5.0	3.6	25	12.5	12.5	12	25
20	C(22)OCO*n*$C_{11}H_{23}$			c	b	a	c	a
21	C(22)OCO*n*$C_{17}H_{35}$	5.0	3.9	a	a	a	a	a
22	C(22)O$COC_{17}H_{33}$ (Oleate)			a	a	a	a	a
23	C(22)O$COC_{17}H_{31}$ (Linoleate)			a	a	a	a	a
24	C(22)OCO$(CH_2)_2COOH$		3.6	a	a	a	50	50
25	C(22)OCO$(CH_2)_3COOH$			a	a	a	100	50
26	C(22)OCO-*cyclohexyl*			3 *gr*	100	25	100	*c*
27	C(22)OCO-*cyclopentyl*			12.5	100	12.5	100	c
28	C(22)OCO*n*C_4H_9			12.5 gr	100	12.5	100	c
29	C(22)OCOPh			a	a	a	100	c
30	C(22)OCOH_2Ph			c	100	a	100	c
31	C(22)OCO*i*Bu			c	100	a	100	c
32	C(22)OCO-*3*-pentyl			50	100	a	100	c
33	C(22)O$COCH_2OPh$			a	a	a	b	c
34	C(22)OCO-*cyclopropyl*			50	100	12.5	b	c
35	C(22)OCO-2-methyl-(*cyclopropyl*)				100	12.5	b	c

[a] The complexation constant K_c (= [*X*-206*M*]/[M^+][X-206]) values were obtained by titration calorimetry (1.2 mM Bu_4NOH/methanol, 25°C, except for compounds **2** and **4** for which pure methanol was used); —infers that no effect was observed during titration.

[b] The test insects were: *Helothis virescens* F (larval stage 1); *Plutella xylostella* L (larval stage 3); *Diabrotica balteata* Lec (larval stage 2); *Frankliniella occidentalis* (mixed) and *Tetranychus urticae* Koch (larvae).

[c] Letters in columns 5–9 have the following significance: (a) Compounds giving <80% mortality at 100 mg litre^{-1} against *H virescens*, *D balteata* and *T urticae* in preliminary tests were not tested further. If inactive against lepidopteran pests in preliminary tests they were not further tested against *P xylostella*. If inactive against *T urticae* in preliminary tests they were not tested against *F occidentalis* in second tests. (b) >100. (c) >50. (gr) Values for *H virescens* are for mortality except where growth inhibition was observed at a lower concentration in which case gr is added to the value quoted. (#) Phytotoxicity to the host plant so severe that reliable insecticidal activity data could not be obtained.

6 Westley JW, *Adv Appl Microbiol* **22**:176–223 (1977).
7 O'Sullivan AC and Struber F *Helv Chim Act* **81**:812–827 (1998).
8 Westley JW, *Polyether Antibiotics*, Vols 1 & 2, Marcel Dekker, New York (1983).
9 Bauer L and Exner O, *Angew Chem* **86**: 419–458 (1974).
10 Anteunis MJ, *Bull Soc Chim Belg* **86**:931–947 (1977).
11 Meerwein H, *Methoden Org Chem* (*Houben-Weyl*) *4th edn* **6**(3):325–365 (1965).
12 Struber F, *Doctoral thesis*, Graz (1996).